American Journal of Medical Genetics 64:107–112 (1996)

Further Linkage Evidence for Localization of Mutational Sites for Nonsyndromic Types of X-Linked Mental Retardation at the Pericentromeric Region

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We used several microsatellite markers scattered along the X chromosome to search for linkage relationships in a large Sardinian pedigree segregating for nonspecific X-linked mental retardation (MRX). Markers DXS573 and AR, located at chromosomal subregions Xp11.4–p11.22 and Xq11.2–q12, respectively, were found to segregate in full concordance with the disease, leading to a LOD score of 4.21 at zero recombination value. Recombination with the disease was found with markers MAOB and DXS454 located at Xp11.4–p11.3 and Xq21.1–q22, respectively; accordingly, markers distal to Xp11.4 and Xq22 also segregated independently of the disease. These findings provide strong linkage evidence in favor of the localization of one MRX mutational site in the pericentromeric region of the human X chromosome, justifying the assignment of a new symbol (MRX26) to our pedigree. Finally, on the basis of the recombinational events observed in the Xq21–q22 region, we have been able to refine the assignment of marker DXS456 to Xq21.33–q22.

KEY WORDS: nonsyndromic XLMR, MRX, genetic linkage

INTRODUCTION

To date, as many as 127 X-linked clinical entities, with mental retardation as a major component, have been described [Neri et al., 1994; Schwartz, 1993]. The overall incidence of X-linked mental retardation (XLMR) has been estimated to be 1.8 per 1,000 males, whereas nearly 2.8 per 1,000 females are expected to be carriers of at least one of the mutations responsible for it [Glass, 1991]. It has been estimated that the fragile-X (FRA-X) syndrome accounts for about one third of all XLMR [Opitz, 1986]. The remaining two thirds have been subdivided into two groups: X-linked mental retardation syndromes (MRXS) and X-linked nonspecific mental retardation (MRX), depending upon the presence or absence of specific clinical manifestations [Neri et al., 1991]. Apart from the FRA-X mutation, whose precise localization at Xq27.3 was confirmed by in situ hybridization studies with closely-linked genes [Szabo et al., 1984; Purrello et al., 1985], tentative chromosomal localizations have been proposed by genetic mapping for most of the syndromal X-linked mental retardations [Mulley et al., 1992]. As expected, attempts to localize nonspecific types of mental retardations by the same methodology have been hampered by the strong limiting factor of having to analyze the relevant segregation data separately for each informative pedigree, including a small number of scorable sibs and mothers with phase unknown. Accordingly, it was decided to assign a progressive (albeit provisional) MRX number only to those pedigrees yielding a LOD score >2.0 at a 0% recombination fraction [Mulley et al., 1992]. Following this approach, at least three X-chromosome locations have been indicated as possible sites of MRX mutations, i.e., Xp21–p22, Xq26–q28, and the pericentromeric region [Mulley et al., 1992]. The latter region is also the one favored by the present linkage study based upon analysis of a large Sardinian pedigree segregating for nonspecific X-linked mental retardation and several genetic markers spanning the X chromosome from Xp22.3–Xq28.

Received for publication June 20, 1995; revision received November 9, 1995.

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PATIENTS AND METHODS

Ascertainment

The large pedigree which is the subject of this report resulted from an extensive population study carried out on the island of Sardinia, with an almost complete ascertainment of males reported to be mentally retarded by the central health service of the regional government. The study led to the identification of 18 unrelated pedigrees segregating for the FRA-X syndrome [Filippi et al., 1983, 1991; Rocchi et al., 1987; Cianchetti et al., 1991, 1992], and 14 pedigrees segregating for either syndromic or nonspecific forms of XLMR without fragile X [Archidiacono et al., 1987]. The three-generation pedigree of the present report belongs to the latter group, and was singled out for linkage investigations because it includes a large number of scorable sibs through two generations. Detailed clinical data on each of the affected members of this pedigree can be found in the published reports mentioned above, where the pedigree is listed as XMR13 [Archidiacono et al., 1987]. The new family code number assigned by the nomenclature committee Genome Database is MRX26.

Molecular Analysis

Twenty ml of peripheral blood were collected, with informed consent, from each subject of the MRX26 family. Genomic DNA was extracted from mononucleated cells following standard procedures, and DNA genotypes were determined by PCR amplification. DNA amplifications by PCR were performed in a 50-μl volume containing 40 ng of genomic DNA, 600 ng of each primer, 250 μM dNTPs, and 1 unit Taq polymerase (Perkin-Elmer) in standard buffer composed of 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5–2.5 mM $MgCl_2$, and 0.001% gelatin. Optimum $MgCl_2$ concentration was determined empirically for each pair of primers. Amplifications were performed in a Perkin-Elmer Cetus thermal cycler (model 480). After an initial denaturation step at 94°C for 120 sec, samples underwent 30 cycles of amplification consisting of 60 sec of denaturation at 94°C, 120 sec of elongation at 72°C, and 10 min of final extension at 72°C for 10 min. Aliquots of 10–15 μl from each amplification product were characterized by electrophoresis on nondenaturing gels of 8% (w/v) polyacrylamide (Bio-Rad Laboratories, Milan, Italy), and the microsatellite (CA) patterns were visualized under UV light, after ethidium bromide staining.

Linkage Analysis

Pairwise segregation analysis of the main character (MRX26) vs. that of each of the informative genetic markers was carried out with the MLINK program of the FASTLINK package (version 2.2) [Lathrop et al., 1985; Cottingham et al., 1993; Schaffer et al., 1994]. A multipoint linkage map was generated with the LINKMAP subprogram of the above-mentioned FASTLINK package. Due to computer limitations, both two-point and multipoint analyses were performed in three different computer runs. Plot of multipoint analysis in the two overlapping regions was generated using the average LOD score value. Allele frequencies for the markers were those reported in the Genome Data Base, assuming fresh mutation rates of zero and full penetrance in affected males. Loci order in the Xq21–q22 region was derived from the program CRIMAP 2.4, which estimates the relative likelihoods of all possible orders and their relative genetic distances.

RESULTS AND DISCUSSION

A complete drawing of the updated pedigree is given in Figure 1. Table I gives a full list of the 14 (CA)n dinucleotide repeat polymorphic loci found to be informative for the present study. Figure 2 shows the segregation of the disease vs. that of 14 microsatellite markers. In Figure 2, the most likely sequence of the loci involved and their combined genetic phase in the two multiple heterozygous mothers (III-2 and IV-2) have been derived from the haplotype segregation observed in the children for individual III-2 and from the available paternal haplotype for individual IV-2. From a quick inspection of Figure 2 it is apparent that: 1) both the obligatory carriers of the MRX26 mutation are heterozygous at marker loci MAOB, DXS573, AR, DXS454, DXS178, and DXS456, one (III-2) at DXS441, DXS1166, DXS1168, and HPRT, and the other (IV-2) at DXS237, PGKP1, DXS453, and DXS458; 2) the main character (MRX26) cosegregates without exception with a block of eight markers encompassing the centromere from Xp11.4 (which is the short-arm distal limit for the location of DXS573)–Xq22 (the long-arm distal limit for the location of DXS458). It is interesting to note that the region between Xp11.4–Xq22 is known to include loci which other studies have proven to recombine fairly frequently with respect to one another even in small sibships [Willems et al., 1993; Gendrot et al., 1994; Robledo et al., unpublished data]. However, on the basis of the LOD score analysis given in Table I, it is clear that the only instances of concordant segregation definitely attributable to close linkage are those involving the comparisons MRX26-DXS573 and MRX26-AR, both yielding a maximum LOD score of +4.21 at zero recombination. In addition, from Figures 1 and 2 and from Table I, it can be seen that the MRX26 mutation has recombined once (in individual V-2) in 15 scorable sibs with respect to marker MAOB (located between Xp11.4–Xp11.3), and twice (in V-2 and V-8) in five scorable sibs with respect to marker DXS237, which is more distally located at Xp22.3. Likewise, direct evidence of recombination between the mutant phenotype and the test markers of the long arm seems to follow an escalating gradient proportional to their physical distance from the centromere, with one recombinant (IV-9) out of 15 scorable sibs with respect to marker DXS454 at Xq21.1–q22, two (IV-5 and IV-9) out of 15 with respect to marker DXS178 at Xq21.33–q22, three (IV-5, IV-9, and IV-13) out of 15 with respect to marker DXS456 at Xq21.33–q22, and four (IV-2, IV-5, IV-9, and IV-13) out of 10 with respect to marker HPRT at Xq26.1. The multipoint linkage analysis illustrated in Figure 3 shows that the highest LOD score value (4.22) is located 0.4 cM distally to marker AR.

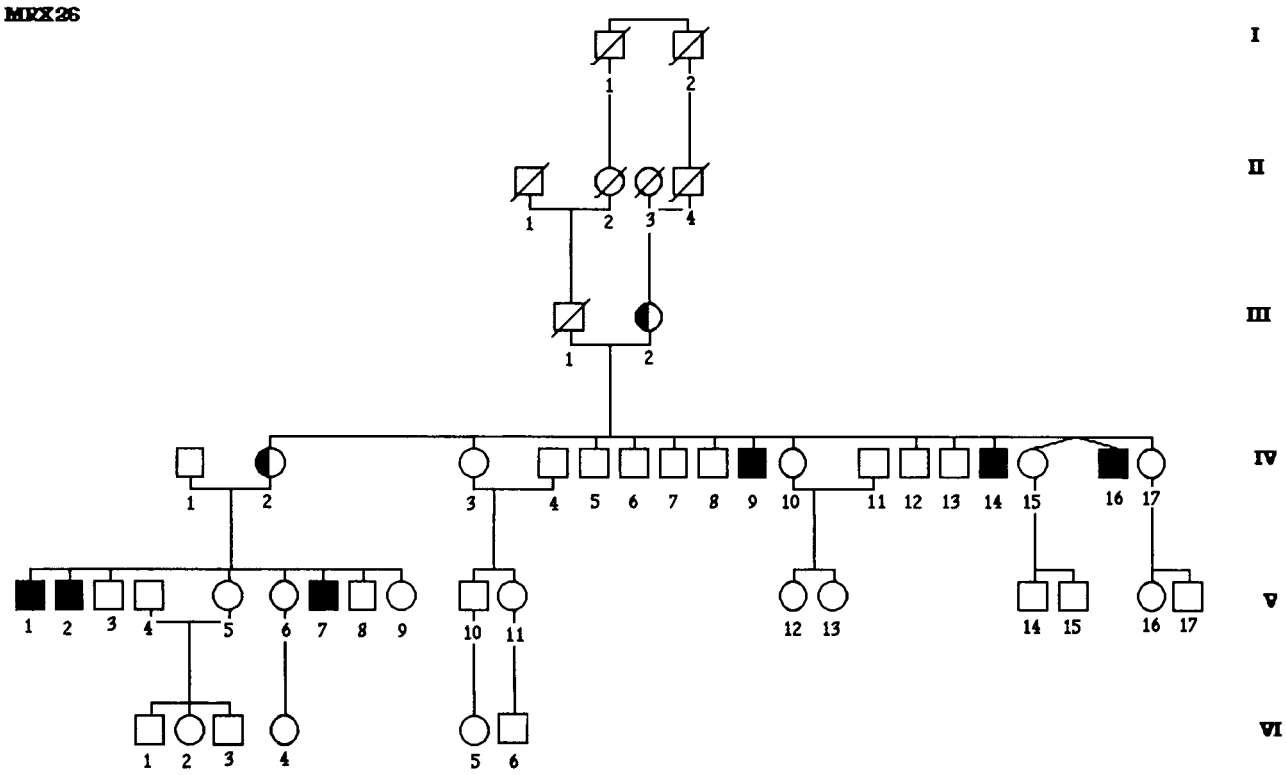

Fig. 1. Pedigree segregating for a nonspecific form of mental retardation. Affected males are symbolized by black squares. Only the individuals belonging to sibships with affected members have been included in linkage studies.

The total number of recombinants directly observed in the region Xp22.3–Xp26.1 seems to be below the theoretical value expected on the assumption of one crossover per 1 million basepairs [Botstein et al., 1980]. In particular, the total absence of recombination in the region Xp11.4–Xq22 raises the possibility that such an effect might be directly related to the nature of the MRX26 mutation as, for instance, would be expected for deletion mutations capable of suppressing the regional crossing-over in heterozygous carriers. To date, several XLMR syndromes have been mapped to the pericentromeric region [Porteous et al., 1992; Wieacker et al., 1987; Watty et al., 1991; Miles and Carpenter, 1989; Schwartz et al., 1990; Saugier-Veber et al., 1993]. This report has localized a nonsyndromic type of mental retardation to the same region, and it would not be at all surprising if several XLMR conditions were eventually proven to be the result of mutations in the same region. Thus, it begins to appear that a great deal of genetic information localized at the pericentromeric re-

TABLE I. Two-Point Linkage Analysis Between MRX-26 Locus and Markers on X Chromosome

		LOD score at theta								
Locus	Location	0.00	0.01	0.05	0.10	0.20	0.30	0.40	Z max	θ max
DXS237	Xp22.3	$-\infty$	−2.51	−1.16	−0.63	−0.18	−0.01	0.04	0.04	0.4
MAOB	Xp11.4–p11.3	$-\infty$	2.15	2.6	2.57	2.16	1.52	0.72	2.6	0.05
DXS573	Xp11.4–p11.22	4.21	4.15	3.88	3.53	2.76	1.89	0.89	4.21	0
AR	Xq11.2–q12	4.21	4.15	3.88	3.53	2.76	1.89	0.89	4.21	0
PGKP1	Xq11.2–q12	1.51	1.48	1.39	1.28	1.02	0.73	0.4	1.51	0
DXS453	Xq12	1.51	1.48	1.39	1.28	1.02	0.73	0.4	1.51	0
DXS441	Xq13.2–q13.3	2.71	2.67	2.49	2.25	1.74	1.16	0.5	2.71	0
DXS1166	Xq13.3–q21.1	2.71	2.67	2.49	2.25	1.74	1.16	0.5	2.71	0
DXS1168	Xq21.1–q21.3	2.71	2.67	2.49	2.25	1.74	1.16	0.5	2.71	0
DXS458	Xq21.1–q22	1.51	1.48	1.39	1.28	1.02	0.73	0.4	1.51	0
DXS454	Xq21.1–q22	$-\infty$	2.15	2.6	2.57	2.16	1.52	0.73	2.6	0.05
DXS178	Xq21.33–q22	$-\infty$	0.16	1.32	1.62	1.56	1.16	0.57	1.62	0.1
DXS456	Xq21.33–q22	$-\infty$	−1.84	0.04	0.67	0.96	0.8	0.44	0.96	0.2
HPRT	Xq26.1	$-\infty$	−3.32	−1.35	−0.61	−0.06	0.07	0.04	0.07	0.3

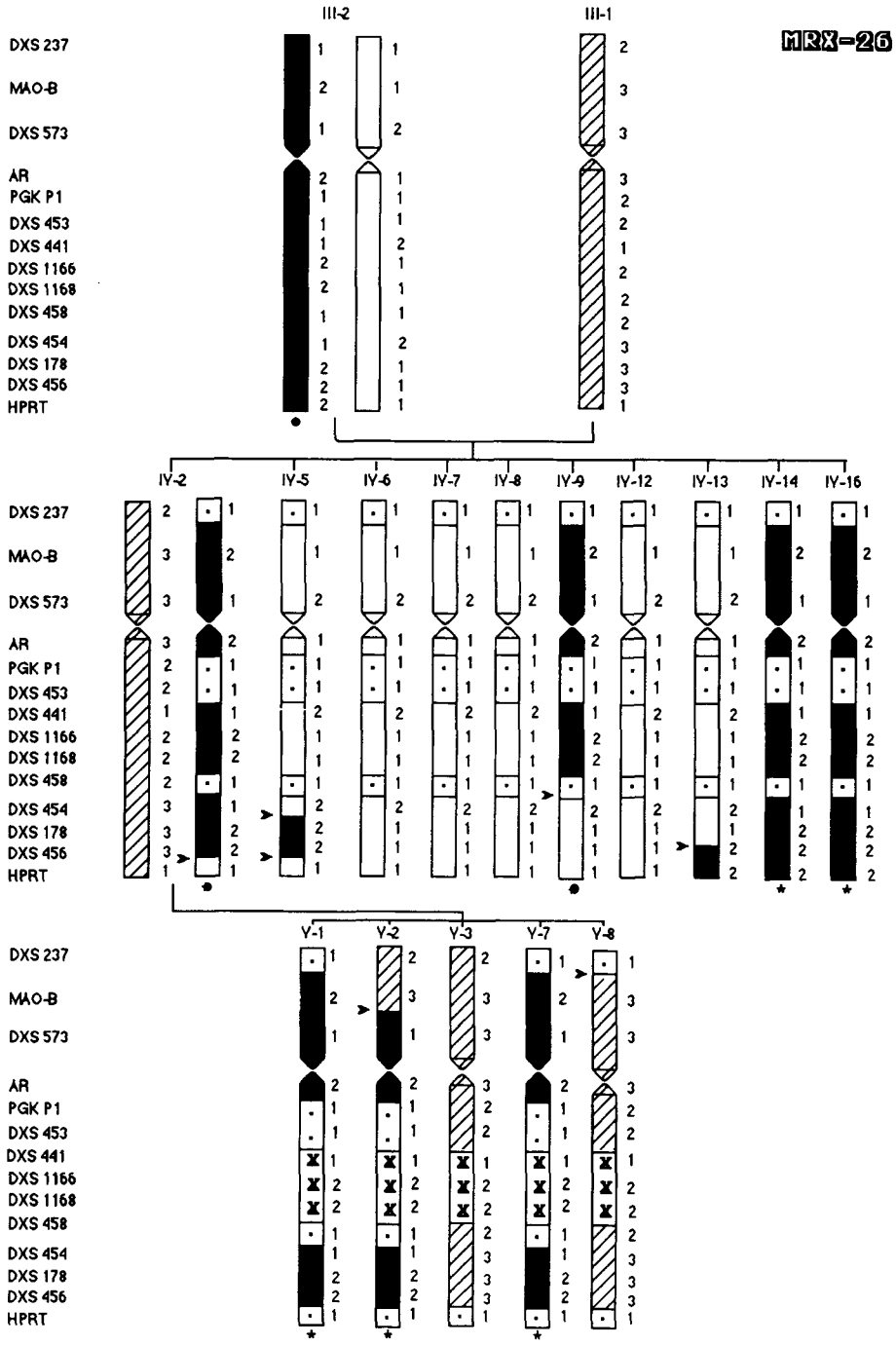

Fig. 2. Segregation of MRX26 mutation vs. 14 dinucleotide repeat markers. Haplotype combination of individual III-2 has been derived from the segregation observed in her 9 male children; asterisk denotes chromosome carrying the disease. X-chromosomal regions, in black from first to last generation, denote portion of the maternal grandmother chromosome which segregated in association with the MRX26 mutation in offspring of multiple heterozygous mothers III-2 and IV-2. Uninformative loci are indicated by dots for III-2 and Xs for IV-2. Arrows indicate crossover events.

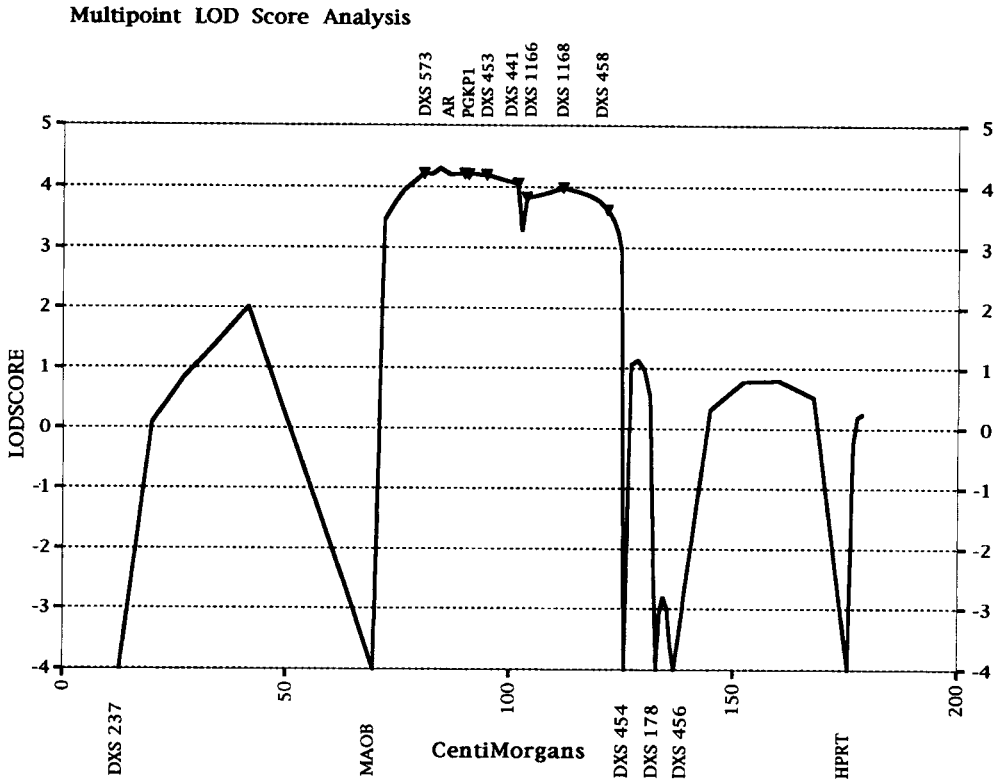

Fig. 3. Multipoint LINKMAP calculations between MRX26 locus and the 14 X-linked markers of Table I.

gion of the X chromosome must be of importance for the normal development of mental capacity, and that a variety of mutational events in this region may trigger the pathogenesis of mental retardation.

In conclusion, the data presented here suggest that the mutation responsible for the type of mental retardation segregating in pedigree MRX26 is located in the pericentromeric region, with upper and lower regional limits at Xp11.4 and Xq22, where the first recombinational events between the disease and more distal informative CA markers were detected. However, the regional localization for the MRX26 mutation is too broad to be of practical help for genetic counselling. Concerning the latter point, we wish to stress the importance of confirming, with a full X-chromosome haplotype segregation analysis, any evidence of linkage, no matter how strong it may be, based on a simple two-point linkage comparison. The justification for such caution is made obvious by a recent finding of our group concerning a large Sardinian pedigree (RENP-1) in which a syndromic type of X-linked mental retardation (Renpenning syndrome) showed an apparent complete linkage relationship (LOD score 3.21 at zero recombination) with respect to two groups of markers (SYN/ARAF-PFC and DXS456-DXS424) which are known to be located, at a nonmeasurable genetic distance, at opposite sides of the centromere [Robledo et al., unpublished data]. Finally, from Figure 2 it is clearly noticeable that the MRX26 mutation has segregated in full concordance with marker DXS458, but that it has recombined at least once with respect to marker DXS454, twice with respect to DXS178, and three times with respect to DXS456. Therefore, our findings are consistent with the proposed order CEN:DXS458:DXS454:DXS178:DXS456:TEL; since marker DXS456 is definitely distal to DXS178, the location of marker DXS456 could be refined to Xq21.33–q22.

ACKNOWLEDGMENTS

We thank Sabrina Lisca for her skillful technical assistance. These investigations were supported by the Italian National Research Council (CNR), Telethon Italy (grant E.201), and the Foundation of Porto Conte Research and Training Laboratories (PCRTL).

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